■■■■ CHAPTER 8

Role of Bradykinin B_1 and B_2 Receptors in Nociception and Inflammation

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8.1 INTRODUCTION

Kinins are believed to be primary mediators of pain and inflammation, acting both to activate nociceptors directly and to induce prolonged inflammatory hyperalgesia. Kinins are released in damaged tissues from bloodborne kininogen precursors by the action of kallikreins. In addition to their algogenic and vasodilator properties,[1,2] kinins trigger an inflammatory positive feedback cycle, stimulating the release of prostaglandins and cytokines, which in turn amplify the responsiveness of inflamed tissue to kinins.[3,4] Elevated levels of circulating bradykinin have been demonstrated in patients with rheumatoid arthritis, confirming the involvement of bradykinin in pathophysiological processes of inflammatory disease.[5] Kinins are also believed to mediate the bone resorption seen in chronic inflammatory conditions such as periodontitis, osteomyelitis, and rheumatoid arthritis.[6] These observations provide a compelling rationale for the development of kinin antagonists as analgesic and anti-inflammatory drugs.

The actions of bradykinin are mediated by means of two distinct receptors designated B_1 and B_2. Both receptors were cloned by Hess and colleagues.[7,8] Bradykinin itself is thought to act mainly through B_2 receptors that are widely and constitutively expressed, for example on immune cells and on neuronal and vascular tissues.[9] The direct activation of B_2 receptors on nociceptors[10] probably explains why intradermal injection of bradykinin is extremely painful in humans.[2,11] Although B_2 receptor antagonists might therefore be

expected to possess analgesic activity, concerns over the safety implications of blocking a putative cardioprotective function of bradykinin[12] has dampened enthusiasm to develop such compounds. The B_1 receptor shows 10- to 50-fold higher affinity for des-Arg[10][kallidin] and for the metabolite des-[Arg[9]]bradykinin than for bradykinin and is expressed in low abundance in normal tissues; the de novo synthesis of B_1 receptors is increased over time following tissue damage and exposure to inflammatory agents.[3,13]

Unlike the B_2 receptor, the ability of B_1 receptor stimulation to activate nociceptors directly has not been firmly established. However, the inducible nature of B_1 receptors at the site of injury may make this a more attractive target for drug development since selective antagonists might be expected to cause minimal disruption of normal physiology in noninflamed tissues and so cause few unwanted side effects. The decision about which bradykinin receptor to target for drug development therefore depends on a careful evaluation of their contributions to painful inflammatory disease and whether their blockade can be achieved without compromising clinical safety.

8.2   PRODUCTION OF KININS IN RESPONSE TO TISSUE INJURY

Kinins are produced in the blood and tissues in response to tissue damage. These include bradykinin, which acts at the B_2 receptor subtype, and des-[Arg[9]]bradykinin and des-[Arg[10]]kallidin, which are B_1 receptor–preferring peptides[5,14,15] (Fig. 8.1). Although the details of the enzymic biosynthesis of bradykinin were known in considerable detail from the work of Rocha e Silva and his colleagues, it was not until 1959 that the identity of bradykinin as a nine-amino acid peptide was revealed.[16] The production of bradykinin from inactive precursors and its rapid degradation in tissues has complicated the study of its pharmacology in vivo.

The production of bradykinin and cognate peptides from their precursor molecules following tissue damage involves serine protease enzymes known generically as kallikreins. Tissue kallikrein and plasma kallikrein are members of different enzyme families,[17,18] but both lead to production of bradykinin. In plasma, activated factor XII cleaves prekallikrein to give active plasma kallikrein, which in turn cleaves high-molecular-weight kininogen to give bradykinin. In the tissues, damage or inflammation leads to release of proteases that cleave prekallikrein to give tissue kallikrein, which then cleaves low-molecular-weight kininogen to give bradykinin and Lys-bradykinin.[17] Production of kinins is stimulated by a variety of inflammatory stimuli and can be evoked by bacterial endotoxins, local irritation (e.g., with uric acid crystals), or antigen/antibody reactions, and degranulating mast cells release proteases that have kallikrein-like activity.[17] It is also significant that the ac-

Tissue   Plasma

Prekallikrein   Prekallikrein

Tissue injury   Blood coagulation cascade

Kallikrein   Kallikrein

Aminopeptidase

Kininogen   Kallidin   Bradykinin   Kininogen

Carboxypeptidase   ACE

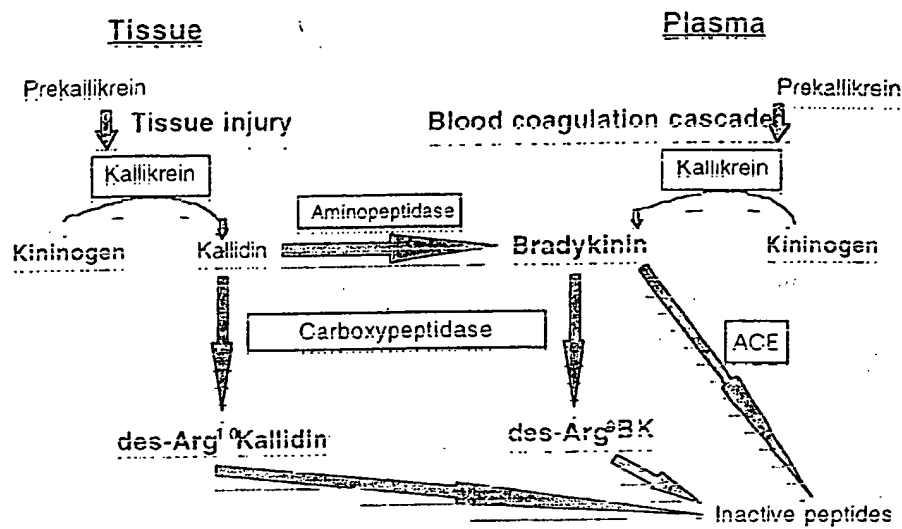des-Arg^{i}-Kallidin   des-Arg^{9}BK

Inactive peptides

Figure 8.1   Production of kinins in response to tissue injury.

tivity of kininases, which normally limit the lifetime of kinins in the biophase, is inhibited by the acid environment of inflamed tissues, thus increasing effective kinin levels. It has also been reported that levels of the B_1 receptor–preferring peptide des-[Arg^9]bradykinin are elevated more than those than those of bradykinin itself during inflammation produced by intradermal carageenan injection.[20]

8.3   ROLE OF BRADYKININ AND OTHER KININS IN PAIN AND INFLAMMATION

Armstrong and his colleagues[21] were the first to show that kinins were produced in an experimental blister on human skin and could cause pain when applied to a blister base. Since pure synthetic bradykinin has been available, it has become readily apparent that this peptide can directly activate nociceptor fibers, innervating many tissues, including skin, joint, muscle, and internal viscera.[19,22-24] Bradykinin is the most potent algogen known and one of the few that stimulates nociceptors in the absence of other chemical agents. Physiologically, it is probably more significant that bradykinin powerfully potentiates the actions of other substances released by tissue inflammation and damage, such as prostaglandins, serotonin, and cytokines[25] (see Ref. 26 for a review). More recently it has been shown that bradykinin is degraded to des-[Arg^9]-bradykinin, which is also an important activator of kinin receptors.

Interestingly, there are tissue-specific differences in the sensitivity of nociceptors to bradykinin. It has been estimated that about half of all unmyelinated and small myelinated afferent fibers in skin are sensitive to excitation by bradykinin (as defined by a 1-min exposure to 10^{-5} M bradykinin), while in deeper tissues over 90% of C fibers may be bradykinin sensitive.[27] It has also been suggested that units that are not overtly sensitive to bradykinin can, nevertheless, show sensitisation of their responses to thermal noxia in the presence of bradykinin.[28] After inflammation with carageenan, more than 80% of cutaneous nociceptors were found to be bradykinin sensitive.[29]

Bradykinin can be shown to produce its excitatory effects on nociceptors by direct depolarization, and recordings from anatomically identified dorsal root ganglion neurones reveals that it is only a proportion of the smaller neurones that are sensitive to bradykinin. Intracellular recordings have shown that the response to bradykinin is through the activation of phospholipase C (PLC). This leads to elevation in intracellular Ca^{2+} and activation of protein kinase C (PKC), and it has been shown that inhibitors of PKC may reduce responses to bradykinin.[30] As the actions of prostaglandins and bradykinin on sensory neurones are additive, the stimulation of prostaglandin production by PLC activation is also relevant.[30] Production of prostaglandins can also be stimulated by bradykinin-induced activation of phospholipase A_2 in non-neuronal cells.[26] The dual action of prostaglandins and bradykinin on nociceptors increases intracellular cyclic AMP levels, which inhibits the slow after-hyperpolarization, thereby increasing the effective burst duration following stimulation.[26] Interaction between kinins and cytokines are also important, in part because of the up-regulation of kinin receptor expression by cytokines[31] (see below).

Indirect effects of bradykinin are important determinants of the effect of tissue inflammation on nociceptor activity (Fig. 8.2). The activation of primary afferent nociceptors and sympathetic neurones leads to release of transmitters contained in the peripheral terminals of these neurones. Neuropeptides released from sensory neurones include substance P, which produces profound neurogenic extravasation plus a smaller amount of vasodilation by activation of NK_1 receptors.[32,33] It is interesting to note that bradykinin-induced bronchoconstriction in asthmatics was reduced by aerosol administration of the NK_1 receptor antagonist FK-224.[34] Release of calcitonin gene–related peptide (CGRP) from the same population of sensory neurones causes vasodilatation and also potentiates the extravasation action of substance P.[32,35] The influence of sympathetic neurone activation and the release of monoamines on inflammation is somewhat controversial. Levine and his colleagues[36,37] have argued for an important role of the sympathetic postganglionic nerve in the production of inflammation and hyperalgesia by bradykinin. In their experiments, activation of the sympathetic nerves, either indirectly by mast cell degranulation or more directly by injection of bradykinin, produced an extravasation response that was decreased by chem-
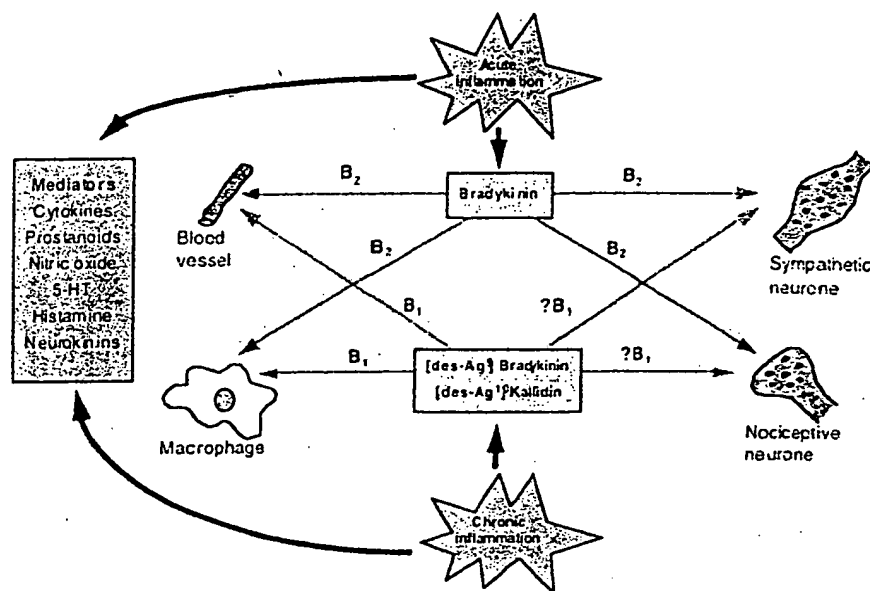
Figure 8.2 Roles of kinins in pain and inflammation. (Modified from Ref. 94.)

ical or surgical sympathectomy.[37,38] Others have conversely found that although peptide depletion with capsaicin or blockade of prostaglandin synthesis with indomethacin would reduce bradykinin-induced hyperalgesia, chemical sympathectomy had no effect.[39,40] Kinins release histamine and serotonin from mast cells and may also be chemotactic and attract immune competent cells to the site of inflammation.[26] It is clear that sensory ganglia are well innervated by postganglionic sympathetic and parasympathetic nerve fibers, however,[41] recent work suggests that there is a direct action of bradykinin that is independent of the sympathetic nerves and a negative feedback operating through the nociceptive afferents and the hypothalomopituitary adrenal axis, which depends on an intact sympathetic system.[42] Bradykinin excites sympathetic ganglion neurones by inhibition of an M-type K^+ current by means of $G_{\alpha q/11}$.[43] Paradoxically, bradykinin can also *inhibit* voltage-dependent N-type Ca^{2+} currents in some cells by a mechanism involving the protein kinase p38-2.[44]

8.4 MOLECULAR PHARMACOLOGY AND SPECIES DIFFERENCES IN BRADYKININ RECEPTORS

The cloning of B_1 and B_2 receptors from human, rat. mouse and rabbit has helped to interpret some of the differences in the pharmacological profiles of B_1 and B_2 receptors across species. For B_1 receptors there are striking species

differences in the affinity of the des-[Arg] metabolites of kallidin and bradykinin, the endogenous ligands for B_1 receptors (and also differences in the affinity of synthetic Leu-substituted analogs of these metabolites). Des-[Arg^{10}]kallidin has much higher affinity than des-[Arg^9]bradykinin for the human B_1 receptor; this difference is less for the rabbit receptor, while the rat and mouse receptors have higher affinity for des-[Arg^9]bradykinin.[45] Furthermore, des-[$Arg^{10}Leu^9$]kallidin and des-[Arg^9Leu^8]bradykinin are partial agonists at the mouse B_1-receptor, but antagonists at the human receptor.[45,46] The affinity and selectivity of ligands for B_1 receptors can also vary across species. For example, B9858, a designer peptide B_1 receptor–selective antagonist, has greater than 1000-fold selectivity for human B_2 over B_2 receptors (0.04 nM compared to 146 nM), whereas affinity for the mouse B_1 receptor is much lower (5.4 nM), and selectively over mouse B_2 receptors is reduced to approximately 30-fold.[45] Overall, the pharmacological profile of human and rabbit B_1 receptors is similar (although there are some minor differences) and contrast to the profiles of rat and mouse receptor.[47–50]

The peptide-binding domains for the B_1 receptor are uncharacterized, although it is known that for peptides the presence of lysl or D-Arg groups at the N terminal are important for affinity, and amino acids at positions 7 and 8 seem to be important for receptor activation and for selectivity over the B_2 receptor, respectively.[51–53] The human B_1 receptor gene is regulated by two promoters and it is tempting to speculate that expression, either constitutive or inducible, is regulated by one or the other of these promoters.[54]

In most species bradykinin is the endogenous ligand for B_2 receptors. Good pharmacological tools are available, including peptide antagonists (e.g., HOE 140) and non-peptide B_2 receptor selective agonists (FR190097) and antagonists (FR167344, FR173657, WIN 64338).[55–57] Species differences exist in the affinity of both peptide and nonpeptide B_2 receptor antagonists. For example, binding studies have shown that FR167344 and FR173657 show higher affinity (subnanomolar) for rat and guinea pig B_2 receptors compared to human B_2 receptors (65 nM).[56,58,59] Unlike the B_1 receptor, the overall pharmacological profile of B_2 receptors across species does not fall conveniently into clear-cut divisions.[60] The peptide-binding domains to the B_2 receptor have been partially characterized using anti-iodiotypic antibodies and site-directed mutagenesis. Extracellular domains 3 and 4 (Fig. 8.3) and the upper regions of transmembrane domains 4 and 7 are important for bradykinin and HOE 140 binding and for receptor activation.[61] Transmembrane 3 (particularly ^{118}Lys) is important for determining peptide selectivity between bradykinin receptor subtypes.[62] Interestingly, sequence alignment of the human, rat, mouse, and rabbit B_2 receptors shows most diversity in the fourth extracellular domain and the upper regions of transmembrane 7, both of which are important for peptide binding. Several polymorphisms of the
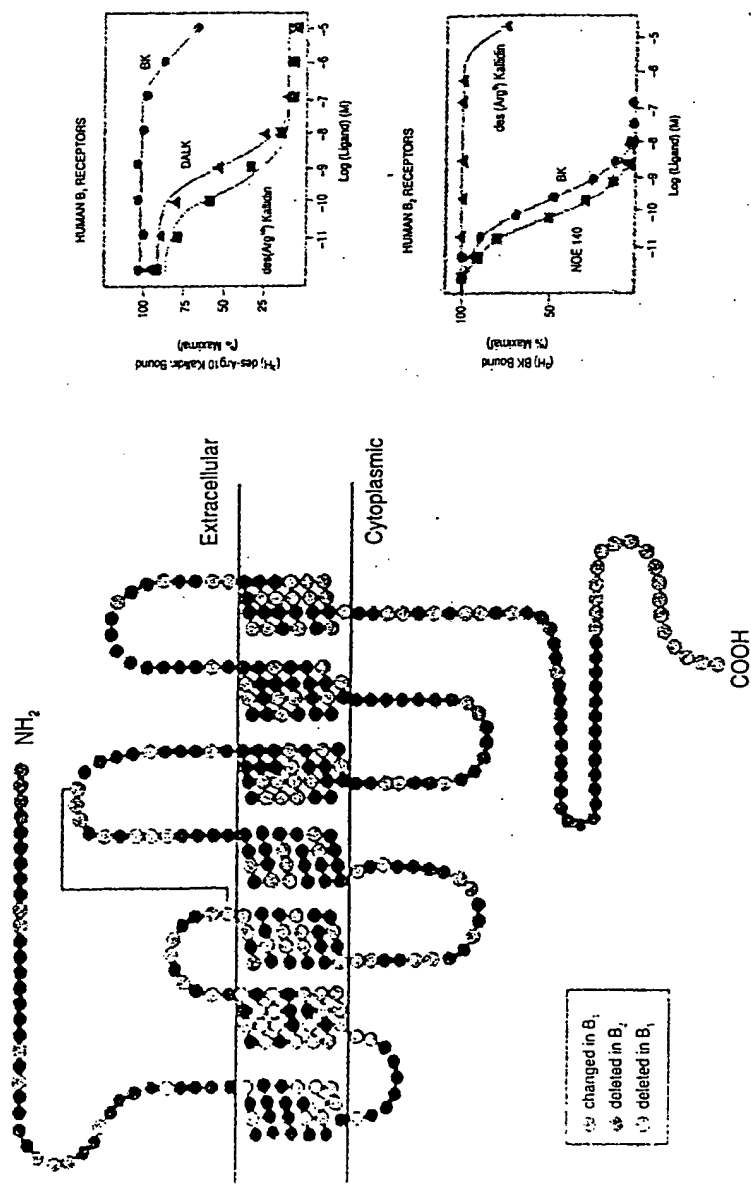
Figure 8.3   Sequence homology of human B_1 and B_2 receptors.

155

human B_2 receptor gene have been identified, including changes to the promotor region and a change resulting in a switch from a tyrosine to a cysteine residue at position 14. The impact of these changes or the involvment of B_2 receptors in hereditary disease is unknown.[63]

The sequence homology between B_1 and B_2 receptors is low (35% at the amino acid level for human B_1 and B_2 receptors[50,60] (Fig. 8.3). Although it has been possible to make peptide dimers with dual affinity for both B_1 and B_2 receptors,[51] it would be challenging to design small synthetic molecules with dual affinity for both B_1 and B_2 receptors. In addition, since bradykinin receptors show similar levels of homology to other G-protein-coupled receptors (e.g., 30% homology to the angiotensin AT_2 receptor), this may limit the level of attainable specificity.

8.5 TISSUE DISTRIBUTION AND EXPRESSION OF BRADYKININ RECEPTORS

The distribution and expression of B_1 and B_2 receptors has recently been reviewed extensively (see Refs. 64 to 66 for detailed descriptions). This review will focus on recent discoveries, particularly those dealing with the distribution of bradykinin receptors in human tissues.

B_1 receptors are often regarded as atypical in that they are not always expressed on the cell surface, and their expression is inducible.[64,66] The induction of B_1 receptors is generally believed to involve de novo synthesis of B_1 receptor protein since it can be inhibited by mRNA and protein synthesis inhibitors such as actinomycin D and cyclohexamide.[64] This is supported by the observation that B_1 receptor induction can lead to an increase in receptor density or number of binding studies, or in an increase in the magnitude of the functional response, but is not always associated with changes in the affinity or potency of B_1 receptor ligands.[8,67]

It is generally assumed that under normal conditions B_1 receptors are not constitutively expressed in tissues, but that expression can be induced or upregulated. In some studies, upregulation is measured by the appearance of specific B_1 receptor sites determined using radioligands or antibodies, and in other studies by development of a functional response to selective B_1 receptor agonists or antagonists. It should be remembered that the appearance of binding sites does not imply that these "receptors" are functional proteins, and on the other hand, the development of a functional response does not exclude the existence of a preformed, nonfunctional receptor protein. Tissue injury can evoke the expression of B_1 receptors and/or functional response. This can be seen in some animal assays, such as persistent hyperalgesia.[26] Importantly, B_1 receptor expression has been detected in inflamed tissues in humans, specifi-

cally in human stomach obtained as biopsy material from patients with gastritis, but not in the same tissue from control patients.[68] Other inducing factors are pharmacological agents including inflammatory mediators such as interleukins, cytokines, or bacterial toxins lipopolysaccharide (LPS) and growth factors.[60,64] The presence of an inflammatory stimulus is not a prerequisite and the induction of a B_1 receptor–mediated responses in isolated tissue preparations can be evoked by incubation in physiological salt solutions (at 37°C).[64,65] The length of the induction period can vary depending on the species; in vivo experiments have shown that induction of B_1 receptor expression evoked by LPS injection takes 2 h in rabbits compared to 12 to 24 h in rats.[13,69] For human isolated tissues (e.g., coronary artery, umbilical vein, and ileum), the length of the induction time varies from 3 h to overnight incubation.[70-72] The up-regulation of B_1 receptor expression is important since it results in amplification of tissue responses to kinins and increased responsiveness to the long-lived des[Arg] metabolites of native kinins.

There are exceptions to the general assumption that in native tissues B_1 receptor–mediated responses cannot be detected except following tissue injury or disease. These exceptions may be species-dependent phenomena. B_1 receptor expression (either inducible or constitutive) has not been reported in guinea pig tissues[60]; no B_1 receptor–mediated responses have been detected in normal tissues from rabbit, rat, pig, or cow.[64-66] In contrast, responses to B_1 receptor agonists have been reported in hemodynamic studies using normal dogs and cats.[64,66,73] Human tissues may also express constitutive B_1 receptors. In human isolated ileum (a tissue that under normal conditions is unresponsiveness to B_1 receptor agonists), protein synthesis inhibitors reduced but did not completely abolish B_1 receptor induction, suggesting the existence of preformed, nonfunctional receptor protein.[70] B_1 receptor immunostaining is present on vascular smooth and endothelial cells in normal human blood vessels and also in vessels with atherosclerotic lesions,[74] although no pharmacological studies were conducted with these vessels to determine receptor functionality. The existence of preformed, nonfunctional receptor protein may explain the apparent short time course for B_1 receptor induction (2 to 3 h for some tissues).[65] This issue should be addressed using a combination of selective B_1 receptor radioligands or antibodies and determination of the absence or presence of a functional B_1 receptor response.

B_2 receptors are regarded as the constitutive bradykinin receptor in that they are expressed in tissues under normal conditions.[60] However, there is some evidence that B_2 receptor expression can also be up-regulated. In dog, cultured tracheal smooth muscle cells incubation (24 h) with forskolin leads to an increase in the number of B_2 receptor binding sites, an effect that can be blocked by protein synthesis inhibitors.[54] Up-regulation of B_2 receptors by interluekin-1 has also been reported for cultured human synovial cells.[75]

B_1 and B_2 receptors are expressed on a wide variety of cell types from different species and these summarized in Table 8.1. Of particular relevance to the role of bradykinin receptors in inflammation and nociception is the presence of both B_1 and B_2 receptors in blood vessels (vascular smooth muscle and endothelial cells) and blood cells (including macrophages). These receptors are involved in the regulation of blood flow and microvascular permeability[64,66] and mediate the extravasation of plasma proteins in response to some inflammatory agents.[65,66] Activation of B_1 and B_2 receptors on macrophages promotes the release of inflammatory mediators (see Fig. 8.2) and at least part of the anti-inflammatory and antinociceptive effects of bradykinin receptor antagonists could be mediated through blockade of receptors on macrophages that have infiltrated the tissue injury or inflammation site. In humans, the recent development of receptor subtype-specific antibodies has allowed the demonstration of B_2 receptors on endothelial and synovial cells and on fibroblasts in synovial membranes from patients with inflammatory joint disease.[76] Surprisingly, no B_1 receptor immunoreactivity was detected in these tissues despite the presence of a chronic inflammatory disease.

TABLE 8.1   Cell Types Expressing B_1 Receptors and B_2 Receptors*

B_1 Receptor Sites/Responses	B_2 Receptor Sites/Responses
Smooth muscle	Smooth Muscle
*Gastrointestinal tract[66,70]	Gastrointestinal tract[66]
*Vascular[66,74,76,77]	*Vascular[66,71,74,76]
Genitourinary tract[66]	*Genitourinary[66]
Airway[66]	Airway[66]
Blood cells	Ocular[66]
*Neutrophils[66,78]	Blood cells
*Macrophages[66,74,76]	*Macrophages[74,76]
Lymphocytes[66]	Neutrophils[78]
*Endothelial cells[66,76,77]	*Endothelial cells[66,76,77]
*Fibroblasts[8,66,74]	*Fibroblasts[8,76]
Neuronal	Neuronal
Superior cervical ganglia[31,79]	Superior cervical ganglia[79]
*CNS (human thalamus, hypothalamus)[10]	*Sensory neurons[6,66,79,80]
Spinal cord (human substantia gelatinosa and interneurons)[77]	CNS (cortex, hypothalamus,
Bone	*caudate, pons, medulla)[77,81]
Osteoclasts[66]	Skeletal muscle[82]
Osteoblasts[66]	*Kidney[66,83]
	*Skin[84]
	*Epithelial cells[85]
	*Synovial cells[76,85,76]
	*Cancer cells[86]

" Data were determined either pharmacologically using functional assays or by radioligand binding and immunocytochemistry with receptor specific antibodies. An asterisk denotes receptors found in human tissues.

It is generally accepted that B_2 receptors are present on nociceptive C and Aδ fibers innervating skin, joint, muscle, tooth pulp, and viscera[26,30] and on postganglionic sympathetic fibers.[26,31,79] Bradykinin activates nociceptors and sensitizes them to other physical and chemical stimuli[26] and activation of B_2 receptors on sympathetic fibers results in blood flow changes and the release of secondary mediators which in turn promote extravasation and/or sensitize nociceptors[26] (see Fig. 8.2). The expression of B_1 receptors on sensory and sympathetic neurones is less clear cut. B_1 receptor agonist des-[Arg^9]bradykinin or des-[Arg^{10}]kallidin failed to activate primary afferents in a spinal nociceptive reflex preparation[30] or cultured rats dorsal root ganglion.[87] No B_1 receptor–mediated responses were detected in dorsal root sensory ganglia (IL1β treated) obtained from either wild-type or B_2 receptor knockout mice, despite the presence of B_1 receptor mRNA.[79] In mouse and rat superior cervical ganglia (sympathetic) B_1 receptor–mediated responses can only be detected following induction with IL1β *and* in the presence of captopril, a peptidase inhibitor.[31,88] These findings suggest that the B_1 receptors that contribute to inflammatory hyperalgesia may not be located on sensory neurones but may be expressed by other cells that release mediators that sensitize or directly activate nociceptors.

There is evidence for a role of central B_2 receptors in blood pressure control and, more relevant to this review, in the processing of nociceptive information. Injection of bradykinin into the brain either intracerebroventricular or intrathecally increases nociceptive behaviors and blood pressure.[26,30] B_2 receptor binding sites (determined using radioligand binding or immunohistochemistry) are present in cerebral cortex, hypothalamus, caudate, subfornical organ, red nucleus, pons, and some nuclei in the medulla (e.g., solitary tract nucleus[26,76,80,81]). Most relevant to nociception are the B_2 receptor sites present in the spinal cord (superficial layers of the dorsal horn and descending noradrenergic bulbospinal neurones[76,80]). Until recently, no B_1 receptor sites were reported in the central nervous system. However, Cassim et al.[76] used immunocytochemical techniques to show B_1 receptors in human brain (hypothalamus and thalamus) and spinal cord (substantia gelatinosa and some interneurones). The role of these B_1 receptors is unknown. The development of improved pharmacological tools for the B_1 receptor, with greater selectivity and metabolic stability, will help to clarify this issue.
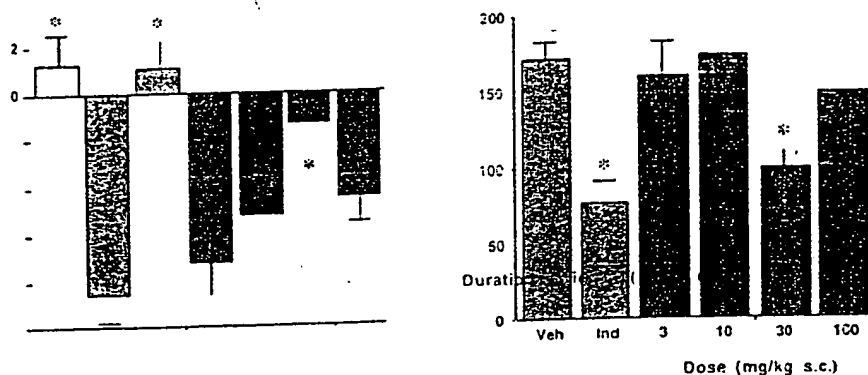
8.6 ANTINOCICEPTIVE ACTIVITY OF PEPTIDE BRADYKININ RECEPTOR ANTAGONISTS

Until recently, the only pharmacological tools that were available to explore the roles of different kinin receptors in pain and inflammation were peptide antagonists, such as the selective bradykinin B_2 receptor antagonist des[Arg^9-

Leu^8]bradykinin, and the B₂ antagonist HOE 140. As will be discussed, there are a number of problems associated with the use of peptide antagonists to elucidate the function of bradykinin receptors in vivo. However, studies using des-Arg^9[Leu^8]bradykinin are consistent with the proposal of an important role of B₁ receptors in inflammatory hyperalgesia. Of particular interest is the ability of des-Arg^9[Leu^8]bradykinin to reverse or prevent the persistent (≥ 24 h) mechanical and thermal hyperalgesia caused by intra-articular injection of Freund's adjuvant, or by ultraviolet irradiation of the skin in rats; in contrast, the B₂ antagonist HOE 140 was ineffective or only weakly active in these assays.[89–91] Moreover, intra-articular injection of the B₁ agonist des-[Arg^9]bradykinin exacerbated hyperalgesia caused by Freund's adjuvant, indicating local induction of B₁ receptors at the site of inflammation.[89,92] Studies of intraarticular plasma extravasation in antigen-induced arthritis also reveal an evolving role for B₁ receptors in the maintenance of chronic inflammation.[93] In other studies examining the effects of ultraviolet irradiation of the paws, thermal hyperalgesia in rats was further increased by intravenous injection of des-[Arg^9]bradykinin and to a lesser extent by bradykinin; this effect of both agonists was reversed by des-Arg^9[Leu^8]bradykinin but not by HOE 140, implicating B₁ rather than B₂ receptors in persistent hyperalgesia.[91] These findings have led to the proposal that stimulation of the B₂ receptor initiates the acute nociceptive and inflammatory response to bradykinin and that the inducible B₁ receptor may play an important role in the development and maintenance of chronic inflammatory hyperalgesia.[94] Consistent with this view is the more resticted activity of peptide B₂ receptor antagonists such as HOE 140 and NPC 567 in acute nociception tests, such as inhibition of acetic acid–induced abdominal constriction[95,96] and tests of carrageenan or urate-induced hyperalgesia.[95,97]

Unfortunately, discrimination of the potentially different roles of B₁ and B₂ receptors from such in vivo studies is compromised by several difficulties. The use of peptide receptor antagonists is problematic because of their metabolic lability and evidence for partial agonist activity. Thus, carboxypeptidases are able to convert certain peptide B₂ receptor antagonists into B₁ receptor blockers,[98] and the B₁ receptor specificity of the antinociceptive effects of des-Arg^9[Leu^8]bradykinin is similarly complicated by its degradation by peptidases in vivo.[99] Peptide antagonists may also behave as partial agonists, as has been reported using in vitro functional assays for des-Arg^9[Leu^8]bradykinin.[45,46,100] An unexplained anomaly is that the dose-response curves for both peptide B₁ and B₂ antagonsits in in vivo assays are typically bell shaped with narrow active dose windows, such that antinociceptive efficacy is lost as the dose is increased"[2,101] (Fig. 8.4). This narrow active dose window makes it difficult to be sure that appropriate doses of the antagonists were evaluated in attempting to determine the relative contributions of B₁ or B₂ receptors in assays of acute or chronic hyperalgesia. Finally,

Carrageenan induced hyperalgesia (rat)   Formalin paw late phase (mouse)

Figure 8.4 Narrow dose window for antinociceptive activity of des-Arg⁹[Leu⁸]bradykinin in the formalin and carrageenan paw tests in mice and rats. Mice received an intraplantar injection of formalin (20 μL of 2.5% solution) 10 min after the test compounds; the duration of licking was recorded throughout the period 10 to 20 min thereafter. In rats, test compounds were administered 2 h after intraplantar injection of carrageenan (4.5 mg) and the change in paw pressure thresholds was determined 1 h later. (From Ref. 92.)

the proposed distinction between B_2 receptor activation in *acute* nociception, compared with the inducible B_1 receptor appearing relatively more important in *chronic* inflammatory hyperalgesia, does not readily account for the antinociceptive and anti-inflammatory activity of des-Arg⁹[Leu⁸]bradykinin in several acute assays, including formalin paw,[92,101,102] capsaicin-induced dermal inflammation,[103] and carrageenan-induced hyperalgesia.[92] In these assays, nociception or inflammation is assessed ≤ 30 min after injection of the inflammatory agent, an observation that is not easily reconciled with the low levels of constitutive expression of the B_1 receptor.

To attempt to overcome the limitations associated with des-Arg⁹[Leu⁸]-bradykinin, we assessed the antinociceptive activity of B9858 (Lys-Lys⁰, Hyp³, Igl⁵, D-Igl⁷, Oic⁸, des-Arg⁹bradykinin), which has recently been described as a potent, stable peptide B_1 receptor antagonist.[52] Unlike Arg⁹[Leu⁸]bradykinin, B9858 behaved as a full antagonist at cloned human and murine B_1 receptors using an in vitro aequorin bioluminescence assay.[45] However, the expectation that B9858 might exhibit a wider antinociceptive dose range compared with des-Arg⁹[Leu⁸]bradykinin in vivo was not borne out in either the carrageenan or formalin paw assays (unpublished observations).

The difficulties associated with the use of peptide antagonists for in vivo functional pharmacology studies underlines the need to develop selective, metabolically stable, nonpeptide B_1 and B_2 receptor antagonists. Recently, the

first orally active nonpeptide B_2 antagonist, FR173657, was described.[104] This compound was found to inhibit bradykinin-induced bronchoconstriction and carrageenan-induced paw edema in rodents but has not yet been evaluated for its effects on nociception. Progress is also being made to develop nonpeptide B_1 antagonists such as the compounds exemplified in a patent filed by Sanofi.

8.7   PHENOTYPE OF $BKr^{-/-}$ MICE

The ability to produce knockout mice with targeted disruption of genes encoding specific proteins has become a popular alternative approach to evaluate the function of neurotransmitter receptors in vivo, particularly where suitable pharmacological antagonists are not yet available. Mice in which the bradykinin B_2 receptor was knocked out $(Bk2r^{-/-})$ have been available for several years, so these have been characterized in most detail. Unlike tissues from wild-type animals, membrane preparations from the ileum or uterus of $Bk2r^{-/-}$ mice provided no detectable binding of [^{3}H]bradykinin, and there were no functional responses to bradykinin in uterine or neuronal tissues.[106]

Recently, we described the behavior of $Bk2r^{-/-}$ mice in a range of conscious animal nociception assays. In view of the known algogenic effects of bradykinin that are believed to be mediated by means of B_2 receptor activation, we observed surprisingly subtle changes in nociception in $Bk2r^{-/-}$ mice.[92] As would be expected following deletion of this gene, $Bk2r^{-/-}$ mice failed to exhibit a nociceptive behavioral response to intraplantar injection of bradykinin (Fig. 8.5), confirming that the direct activation of nociceptors by bradykinin agonists involves the B_2 rather than the B_1 receptor, at least in non-inflamed tissues. These findings are consistent with the ability of HOE 140 and other peptide B_2 receptor antagonists to inhibit nociception elicited by bradykinin in animals and humans.[2,95,96] The $Bk2r^{-/-}$ mice also failed to develop paw edema or thermal hyperalgesia in response to intraplantar injection of carrageenan, indicating that the action of bradykinin at B_2 receptors is an essential initial step in the inflammatory response to this polysaccharide. This observation is consistent with the inhibition of carrageenan-induced edema and thermal hyperalgesia by peptide B_2 antagonists such as NPC 567.[97]

In contrast to their abnormal response to bradykinin and to carrageenan, $Bk2r^{-/-}$ mice exhibited intact nociceptive responses in a number of other tests. Their spinal nociceptive reflexes, assessed using a paw flick test, appeared normal, suggesting that bradykinin is not involved in the activation of thermal nociceptors in the absence of inflammation or tissue damage. Moreover, nociception and inflammatory hyperalgesia elicited by intraplantar injection of formalin or complete Freund's adjuvant were indistinguishable in $Bk2r^{-/-}$ and wild-type mice (Fig. 8.5). This finding was unexpected in view of reports that

Intraplantar injection of bradykinin   Late phase response to formalin
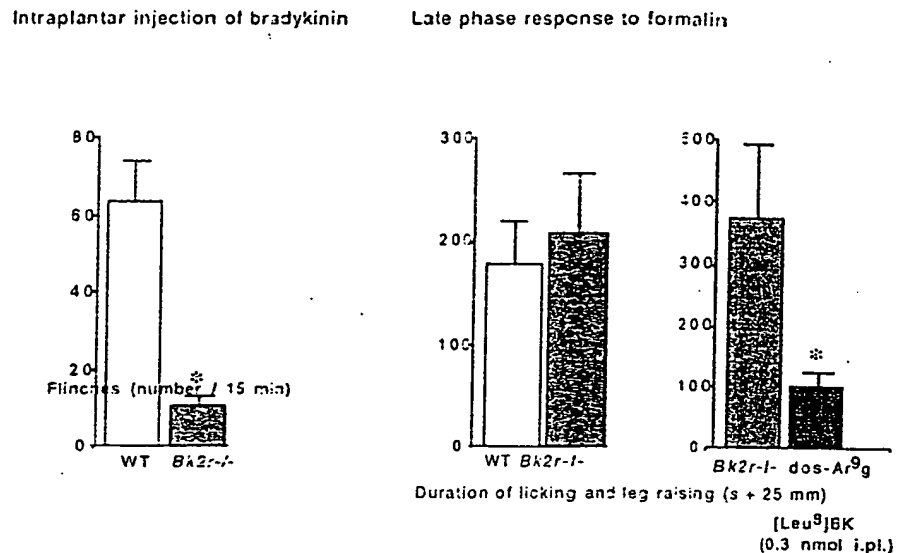
Figure 8.5   Response of *Bk2r⁻* mice to intraplantar injection of bradykinin or formalin. Aversive behaviors elicited by intraplantar injection of bradykinin (10 nmol) or formalin (2.5%) were recorded by direct observation for up to 35 min. Also shown is the inhibition of the late-phase response to formlin by intraplantar injection of des-Arg⁹[Leuˢ]bradykinin (0.3 nmol) in *Bk2r⁻* mice. (From Ref. 92.)

peptide B_2 receptor antagonists such as HOE 140 and NPC 567 could attenuate nociceptive responses evoked by formalin[101] and increase the load tolerated by the arthritic joints of experimental rats.[107] Interestingly, the antinociceptive effect of the B_1 receptor antagonist des-Arg⁹[Leuˣ]bradykinin against the late-phase response to formalin was still demonstrable in *Bk2r⁻* mice (Fig. 8.5), indicating that induction of B_1 receptors is not dependent on B_2 receptor stimulation. The findings from phenotypic characterization of *Bk2r⁻* mice suggests that B_2 receptor activation is required for some, but not all, responses to noxious stimuli and that the clinical potential of B_2 receptor antagonists to treat chronic pain and inflammation may therefore be limited.

The first bradykinin B_1 receptor knockout mice (*Bk1r⁻*) have been generated only recently; unlike wild-type animals, administration of bacterial lipopolysaccharide to *Bk1r⁻* mice failed to increase B_1 receptor mRNA in peripheral tissues (heart, lungs, kidney, liver, ileum, and stomach). In isolated tissue preparations (stomach and ileum) the contractile response to the B_1 receptor agonist des-[Arg⁹]bradykinin was absent in *Bk1r⁻* mice.[108] Such animals provide a means to circumvent the limitations of currently available peptide bradykinin antagonists to clarify the relative contributions of the B_1 and B_2 receptors to acute and chronic noxious and inflammatory responses. The

phenotype of $Bk1r^{-/-}$ mice in assays of acute and chronic inflammatory hyperalgesia has not yet been characterized; this remains an important objective for future investigations.

8.8 B₁ OR B₂ RECEPTORS AS DRUG DEVELOPMENT TARGETS

The existence of two bradykinin receptors begs the question of which is the better target for drug development. The low (35%) sequence homology between the B₁ and B₂ receptors suggests that it would be difficult to design a pharmacological antagonist that would block both of these and still retain adequate selectivity over other G protein–coupled receptors. Faced with the need to choose one receptor as a target for development, it is important to assess which of these is the most compelling.

The evidence summarized in Table 8.2 indicates that at present the B₁ receptor remains an attractive target because it is induced locally at the site of tissue injury, so that blocking this receptor should cause minimal disturbance of physiology in normal tissues, and because studies using peptide B₁ receptor antagonists indicate that these are active in a wide range of nociception assays involving both acute and chronic inflammation. Although the response of $Bk1r^{-/-}$ mice to noxious stimulation has not yet been assessed, these animals appear healthy, and no adverse consequence of blocking or deleting the B₁ receptor is known at present. In contrast to this are a number of emerging reservations about the B₂ receptor as an analgesic drug target. Pharmacological blockade of the B₂ receptor results in antinociception in only a narrow range of animal assays compared with B₁ antagonists. While recognizing the limitations of studies using peptide antagonists, it is clear that the subtle changes in response to noxious stimuli seen in $Bk2r^{-/-}$ mice are broadly consistent with this conclusion.

A second important area of concern regarding the development of B₂ receptor antagonists involves the ubiquitous and constitutive expression of these receptors that may give rise to adverse effects, particularly involving the

TABLE 8.2   B₁ or B₂ Receptor: Which Is the Best Drug Target?

	B₁	B₂
Antinociceptive activity of peptide antagonists	Wide range of acute and chronic assays	Limited activity in acute assays
Phenotype of $Bkr^{-/-}$ mice	Not known	Respond to most noxious stimuli
Expression	Inducible	Constitutive
Potential harmful effects	None known	Cardioprotection? Hypertension?

cardiovascular system. A major issue surrounds the potential for provoking or exacerbating hypertension by blocking B_2 receptors. The kallikrein-kinin system regulates water and sodium excretion and so participates in blood pressure homeostasis. Although $Bk2r^{-/-}$ mice exhibit normal resting blood pressure, recent studies have demonstrated that the hypertensive response to a high-salt diet was almost doubled in $Bk2r^{-/-}$ mice compared with wild-type controls.[109,110] These observations suggest that drugs blocking the B_2 receptor might predispose individuals to develop salt-sensitive hypertension and may counteract the beneficial effects of antihypertensive therapies. The contribution of B_1 receptor–mediated events to blood pressure homeostasis has not yet been investigated under these conditions. Another major clinical safety concern is that the B_2 receptor antagonist HOE 140 has been shown to attenuate the antiarrhythmic (cardioprotective) effects of ischemic preconditioning in anesthetized dogs.[12] Again, the consequences of blocking the B_1 receptor have not been established under these conditions.

8.9   CONCLUSIONS AND FUTURE DIRECTIONS

There is a strong preclinical rationale for the development of bradykinin antagonists to treat pain and inflammation. Both B_1 and B_2 receptors represent potential analgesic drug targets, although the B_1 receptor is currently of most interest because it may provide greater clinical efficacy in chronic pain states and a more favorable clinical safety profile. The question of whether bradykinin antagonists can be developed successfully into safe and effective anti-inflammatory analgesic drugs awaits the identification of nonpeptide antagonists that have high affinity for the human receptor and careful preclinical safety and efficacy studies in appropriate species. Studies to characterize the expression and localization of bradykinin in normal and pathological tissues will also make an important contribution to our understanding of their role in health and disease.

REFERENCES

1. Coffman, J. (1966). The effect of aspirin on pain and hand blood flow responses to intra-arterial injection of bradykinin. Clin. Pharmacol. Ther. 7:26–37.
2. Whalley, E. T., Clegg, S., Stewart, J. M., and Vavrek, R. J. (1987). The effect of kinin agonists and antagonists on the pain response of the human blister base. Arch. Pharmacol. 336:652–655.
3. Regoli D., and Barabe, J. (1980). Pharmacology of bradykinin and related kinins. Pharmacol. Rev. 32:1–46.

4. Burch, R. M., Connor, J. R., and Tiffany, C. W. (1989). The kallikrein–kininogen–kinin system in chronic inflammation. Agents Actions 27:258–260.

5. Hargreaves, K. M., Troullos, E., Dionne, R. A., Schmidt, E. A., Schafer, S., and Joris, J. L. (1988). Bradykinin is increased during acute and chronic inflammation: therapeutic implications. Clin. Pharmacol. Ther. 6:613–621.

6. Ljunggren, O., and Lerner, U. H. (1990). Evidence for BK₁ bradykinin receptor-mediated prostaglandin formation in osteoblasts and subsequent enhancement of bone resorption. Br. J. Pharmacol. 101:382–386.

7. Hess, J. F., Borkowski, J. A., Young, G. S., Strader, C. D., and Ransom, R. W. (1992). Cloning and pharmacological characterisation of a human bradykinin (BK-2) receptor. Biochem. Biophys. Res. Commun. 184:260–268.

8. Menke, J. G., Borkowski, J. A., Bierilo, K. K., MacNeil, T., Derrick, A. W., Schneck, K. A., Ransom, R. W., Strader, C. D., Linemeyer, D. L., and Hess, J. F. (1994). Expression cloning of a human B₁ bradykinin receptor. J. Biol. Chem. 269:21583–21586.

9. Sharif, N. A., and Whiting, R. L. (1991). Identification of B₂-bradykinin receptors in guinea-pig brain regions, spinal cord and peripheral tissues. Neurochem. Int. 18:89–96.

10. Steranka, L. R., Manning, D. C., de Haas, C. J., Ferkany, J. W., Borosky, S. A., Connor, J. R., Vavrek, R. J., and Stewart, J. M. (1988). Bradykinin as a pain mediator: receptors are localized to sensory neurons, and antagonists have analgesic actions. Proc. Natl. Acad. Sci. USA 85:3245–3249.

11. Manning, D. C., Srinivasa, N. R., Meyer, R. A., and Campbell, J. N. (1991). Pain and hyperalgesia after intradermal injection of bradykinin in humans. Clin. Pharmacol. Ther. 50:721–729.

12. Vegh, A., Papp, J. G., and Parratt, J. (1994). Attenuation of the antiarrhythmic effect of ischaemic preconditioning by blockade of bradykinin B₂ receptors. Br. J. Pharmacol. 113:1167–1172.

13. Regoli, D. C., Marceau, F., and Lavigne, J. (1981). Induction of B₁ receptors for kinins in the rabbit by a bacterial lipopolysaccharide. Eur. J. Pharmacol. 71:105–115.

14. Barlas, A., Sugio, K., and Greenbaum, L. A. (1985). Release of T-kinin and bradykinin in carageenin induced inflammation in the rat. FEBS Lett. 190:268–270.

15. Farmer, S. G. (1997). The handbook of immunopharmacology: the kinin system, Academic Press, San Diego, Calif.

16. Boissonas, R. A., Guttman, S. T., and Jaquenoud, P. A. (1960). Synthèse de la L-arginyl-L-prolyl-L-prolyl-glycyl-L-phenylanalyly-L-seryl-L-phenylalanyl-L-arginine: distinction entre cet octapeptide et la bradykinine. Helv. Chim. Acta. 43:1481–1487.

17. Bhoola, K. D., Figueroa, C. D., and Worthy, K. (1992). Bioregulation of kinins: kallikreins, kininogens and kininases. Pharmacol. Rev. 44:1–80.

18. Clements, J. A. (1997). The molecular biology of the kallikreins and their roles in inflammation. In: The handbook of immunopharmacology: the kinin system, ed. Farmer, S. G., Academic Press, San Diego, Calif., pp. 72–97.

19. Kress, M., and Reeh, P. W. (1996). Chemical excitation and sensitization in no-ciceptors. In: Neurobiology of nociceptors, ed. Belmonte, C., and Cervero, F., Oxford University Press, Oxford, pp. 258–297.

20. Burch, R. M., and DeHaas, C. (1990). A bradykinin antagonist inhibits car-rageenan edema in rats. Naunyn Schmiedebergs Arch. Pharmacol. 342: 189–193.

21. Armstrong, D., Dry, R. M. L., Keele, C. A., and Markham, J. M. (1952). Pain-producing substances in blister fluid and serum. J. Physiol. 117:4P.

22. Handwerker, H. O. (1976). Influence of algogenic substances and pros-taglandins on discharges of unmyelinated cutaneous nerve fibres identified as nociceptors. In: Advances in pain research and therapy, ed. Bonica, J. J., and Albe-Fessard, D. G., Raven Press, New York, pp. 41–51.

23. Kumazawa, T., and Mizumura, K. (1980). Chemical responses of polymodal re-ceptors of the scrotal contents in dogs. J. Physiol. 299:219–231.

24. Schaible, H.-G., and Grubb, B. D. (1993). Afferent and spinal mechanisms of joint pain. Pain 55:5–54.

25. Dray, A. (1994). Tasting the inflammatory soup: role of peripheral neurones. Pain Rev. 1:153–171.

26. Dray, A., and Perkins, M. (1997). Kinins and pain. In: The handbook of im-munopharmacology: the kinin system, ed. Farmer, S. G., Academic Press, San Diego, Calif., pp. 157–172.

27. Lang, E., Novak, A., Reeh, P. W., and Handwerker, H. O. (1990). Chemosensitivity of fine afferents from rat skin in vitro. J. Neurophysiol. 63:887–901.

28. Beck, P. W., and Handwerker, H. O. (1974). Bradykinin and serotonin effects on various types of cutaneous nerve fibres. Pflugers Arch. 347:209–222.

29. Kirchoff, C. G., Jung, S., Reeh, P. W., and Handwerker, H. O. (1990). Carrageenan inflammation increases bradykinin sensitivity of rat cutaneous no-ciceptors. Neurosci. Lett. 111:206–210.

30. Dray, A., Patel, I. A., Perkins, M. N., and Rueff, A. (1992). Bradykinin-induced activation of nociceptors: receptor and mechanistic studies on the neonatal rat spinal cord-tail preparation in vitro. Br. J. Pharmacol. 107:1129–1134.

31. Seabrook, G. R., Bowery, B. J., and Hill, R. G. (1995). Bradykinin receptors in mouse and rat isolated superior cervical ganglion. Br. J. Pharmacol. 115:368–372.

32. Brain, S. D., and Williams, T. J. (1985). Inflammatory oedema induced by syn-ergism between calcitonin gene related peptide and mediators of increased vas-cular permeability. Br. J. Pharmacol 86:855–860.

33. Shepheard, S. L., Williamson, D. J., Williams, J., Hill, R. G., and Hargreaves, R. J. (1995). Comparison of the effects of sumatriptan and the NK, antagonist

CP-99,994 on plasma extravasation in dura mater and c-fos mRNA expression in trigeminal nucleus caudalis of rats. Neuropharmacology 34:255–261.

34. Ichinose, M., Nakajima, N., Takahashi, T., Yamauchi, H., Inoue, H., and Takishima, T. (1992). Protection against bradykinin-induced bronchoconstriction in asthmatic patients by neurokinin receptor antagonist. Lancet 340: 1248–1251.

35. Williamson, D. J., Hargreaves, R. J., Hill, R. G., and Shepheard, S. L. (1997). Sumatriptan inhibits neurogenic vasodilation of dural blood vessels in the anaesthetized rat: intravital microscope studies. Cephalalgia 17:525–531.

36. Taiwo, Y. O., Heller, P. H., and Levine, J. D. (1990). Characterisation of distinct phospholipases mediating bradykinin and noradrenaline hyperalgesia. Neuroscience 39:523–531.

37. Basbaum, A. I., and Levine, J. D. (1991). The contribution of the nervous system to inflammation and inflammatory disease. Can. J. Physiol. Pharmacol. 69:647–651.

38. Coderre, T. J., Chan, A. K., Helms, C., Basbaum, A. I., and Levine, J. D. (1991). Increasing sympathetic nerve terminal-dependent plasma extravasation correlates with decreased arthritic joint injury in rats. Neuroscience 40:185–189.

39. Koltzenburg, M., Kress, M., and Reeh, P. W. (1992). The nociceptor sensitisation by bradykinin does not depend on sympathetic neurons. Neuroscience 46:465–473.

40. Schuligoi, R., Donnerer, J., and Amann, R. (1994). Bradykinin-induced sensitization of afferent neurons in the rat paw. Neuroscience 59:211–215.

41. Kummer, W. (1994). Sensory ganglia as a target of autonomic and sensory nerve fibres in the guinea pig. Neuroscience 59:739–754.

42. Green, P. G., Janig, W., and Levine, J. D. (1997). Negative feedback neuroendocrine control of inflammatory response in the rat is dependent on the sympathetic postganglionic neuron. J. Neurosci. 17:3234–3238.

43. Jones, S., Brown, D. A., Milligan, G., Willer, E., Buckley, N. J., and Caulfield, M. P. (1995). Bradykinin excites rat sympathetic neurons by inhibition of M currents through a mechanism involving B_2 receptors and $G_{aq'11}$. Neuron, 64:394–405.

44. Wilk-Blaszczak, M. A., Stein, B., Xu, S., Barbosa, M. S., Cobb, M. H., and Belardetti, F. (1998). The mitogen-activated protein kinase p38-2 is necessary for the inhibition of N-type calcium current by bradykinin. J. Neurosci. 18:112–118.

45. MacNeil, T., Feighner, S., Hreniuk, D. L., Hess, J. F., and Van der Ploeg, L. H. T. (1997). Partial agonists and full antagonists at the human and murine bradykinin B_1 receptors. Can. J. Physiol. Pharmacol. 75:735–740.

46. Allogho, S. N., Gobeil, F., Pheng, L. H., Nguyen-Le, X. K., Neugebauer, W., and Regoli, D. (1995). Kinin B_1 and B_2 receptors in mouse. Can. J. Physiol. Pharmacol. 73:1759–1764.

47. Gobeil, F., Neugebauer, W., Nguyen-Le, X. K., et al. (1997). Pharmacological profiles of the human and rabbit B_1 receptors. Can. J. Physiol. Pharmacol. 75:591–595.

48. Brown, M., Weeb, M., Phillips, E., Skidmore, E., and McIntyre, P. (1995). Molecular studies on kinin receptors. Can. J. Physiol. Pharmacol. 73:780–786.

49. Hess, J. F., Borkowski, J. A., MacNeil, T., et al. (1994). Differential pharmacology of cloned human and mouse B_2 bradykinin receptors. Mol. Pharmacol. 45:1–8.

50. Hess, J. F. (1997). Molecular pharmacology of kinin receptors. In: The handbook of immunopharmacology: the kinin system, ed. Farmer, S. G., Academic Press, San Diego, Calif., pp. 45–56.

51. Gera, L., Stewart, J. M., Whalley, E. T., Burkard, M., and Zuzack, J. S. (1996). A new class of potent bradykinin antagonist dimers. Immunopharmacol 33:178–182.

52. Stewart, J. M., Gera, L., Hanson, W., Zuzack, J. S., Burkard, M., McCullough, R., and Whalley, E. T. (1996). A new generation of bradykinin antagonists. Immunopharmacol 33:51–60.

53. Reissmann, S., Schwuchow, C., Seyfarth, L., et al. (1996). Highly selective bradykinin agonists and antagonists with replacement of proline residues by N-methyl-D- and L-phenylalanine. J. Med. Chem. 39:929–936.

54. Yang, X., and Polgar, P. (1996). Genomic structure of the human bradykinin B_1 receptor gene preliminary characterisation of its regulatory regions. Biochem. Biophys. Res. Commun. 222:718–725.

55. Aramori, I., Zenhok, J., Morikawa, N., et al. (1997). Nonpeptide mimic of bradykinin with long-acting properties at the bradykinin B_2 receptor. Mol. Pharmacol. 52:16–20.

56. Aramori, I., Zenhok, J., Morikawa, N., et al. (1997). Novel subtype-selective nonpeptide bradykinin receptor antagonists FR16344 and FR173657. Mol. Pharmacol. 51:171–176.

57. Scherrer, D., Daeffler, L., Trifilieff, A., and Gies, J.-P. (1995). Effects of WIN64338, a nonpeptide bradykinin B_2 receptor antagonist, on guinea-pig trachea. Br. J. Pharmacol. 11:1127–1128.

58. Inamura, N., Asano, M., Hatori, C., et al. (1997). Pharmacological characterisation of a novel, orally active, nonpeptide bradykinin B_2 receptor antagonist, FR 167344. Eur. J. Pharmacol. 333:79–86.

59. Griesbacher, T., Sametz, W., Legat, F. J., et al. (1997). Effects of a non-peptide B_2 antagonist FR173657 on kinin-induced smooth muscle contraction and relaxation, vasoconstriction and prostaglandin release. Br. J. Pharmacol. 121:469–476.

60. Regoli, D., Rizzi, A., Calo, G., Nsa Allogho, S., and Gobeil, F. (1997). B1 and B2 kinin receptors in various species. Immunopharmacol. 36:143–147.

61. Abd Alla, S., Quitterer, U., Grigoriev, S., et al. (1997). Extracellular domains of the bradykinin B_2 receptor involved in ligand binding and agonist sensing defined by antipeptide antibodies. J. Biol. Chem. 271:1748–1755.

62. Fathy, D. B., Mathis, S. A., Leeb, T., and Leeb-Lundberg, L. M. F. (1998). The third transmembrane domains of the human B1 and B2 bradykinin receptors subtypes are involved in discriminating between subtype-selective ligands. J. Biol. Chem. 273: 12210–12218.

63. Braun, A., Kammerer, S., Bohme, E., Muller, B., and Roscher, A. A. (1995). Identificantion of polymorphic sites of the human bradykinin B₂ receptor gene. Biochem. Biophys. Res. Commun. 211:234–240.

64. Marceau, F. (1995). Kinin B₁ receptors: a review. Immunopharmacol 30:1–26.

65. Marceau, F. (1997). Kinin B₁ receptor induction and inflammation. In: The handbook of immunopharmacology: the kinin system, ed. Farmer, S. G., Academic Press, San, Diego, Calif., pp. 143–156.

66. Hall, J. M., and Morton, I. K. M. (1997). The pharmacology and imunopharmacology of kinin receptors. In: The handbook of immunopharmacology: the kinin system, ed. Farmer, S. G., Academic Press, San Diego, Calif., pp. 9–44.

67. Drummond, G. R., and Cocks, T. M. (1995). Endothelium-dependent relaxations mediated by inducible B₁ and constitutive B₂ kinin receptors in the bovine isolated coronary artery. Br. J. Pharmacol. 116:2473–2481.

68. Bhoola, R., Ramsaroop, R., Naidoo, S., Muller-Esterl, W., and Bhoola, K. D. (1997). Kinin receptor status in normal and inflamed gastric mucosa. Immunopharmacol 36:161–166.

69. Tokumasu, T., Veno, A., and Oh-ishi, S. (1995). A hypotensive response induced by des-Arg⁹-bradykinin in young brown/Norway rats pretreated with endotoxin. Eur. J. Pharmacol. 274:225–228.

70. Zuzack, J. S., Burkard, M. R., Cuadrado, D. K., Greer, R. A., Selig, W. M., and Whalley, E. T. (1996). Evidence of a bradykinin B1 receptor in human ileum: pharmacological comparison to the rabbit aorta B1 receptor. J. Pharmacol. Exp. Ther. 227:1337–1343.

71. Gobeil, F., Pheng, L. H., Badini, I., et al. (1996). Receptors for kinins in the human isolated umbilical vein. Br. J. Pharmacol. 118:289–294.

72. Drummonds, G. R., and Cocks, T. M. (1995). Endothelium-dependent relaxation to the B₁ kinin receptor agonist des-Arg⁹-bradykinin in human coronary arteries. Br. J. Pharmacol. 116:3083–3085.

73. Santiago, J. A., Garrison, E. A., Champion, H. C., et al. (1995). Analysis of responses to kallidin, DABK, and DAK in feline hindlimb vascular bed. Am. J. Physiol. 269:H2057–H2064.

74. Raidoo, D. M., Ramsaroop, R., Naidoo, S., Muller-Esterl, W., and Bhoola, K. D. (1997). Kinin receptors in human vascular tissue: their role in atheromatous disease. Immunopharmacol 36:153–160.

75. Bathon, J. M., Manning, D. C., Goldman, D. W., Towns, M. C., and Proud, D. (1992). Characterisation of kinin receptors on human synovial cells and upregulation of receptor number by interleukin-1. J. Pharmacol. Exp. Ther. 260: 384–392.

76. Cassim, B., Naidoo, S., Ramsaroop, R., and Bhoola, K. D. (1997). Immunolocalisation of bradykinin receptors on human synovial tissue. Immunopharmacol 36:121–126.

77. Raidoo, D. M., and Bhoola, K. D. (1997). Kinin receptors on human neurones. J. Neuroimmunol. 77:39–44.

78. Carl, V. S., Moore, E. E., Moore, F. A., and Whalley, E. T. (1996). Involvement of bradykinin B1 and B2 receptors in human PMN elastase release. Immunopharmacol, 33:325–329.

79. Seabrook, G. R., Bowery, B. J., Heavens, R., et al. (1997). Expression of B_1 and B_2 bradykinin receptors mRNA and their functional role in sympathetic gnaglia and dorsal root sensory ganglia neurones from wild-type and B_2 receptor knockout mice. Neuropharmacol 36:1009–1017.

80. Lopes, P., Kar, S., Chretien, L., Regoli, D., Quirion, R., and Couture, R. (1995). Quantitative autoradiographic localization of $[^{125}I\text{-}Tyr^8]$bradykinin receptor binding sites in the rat spinal cord: effects of neonatal capsaicin, noradrenergic deafferentation, dorsal rhizotomy and peripheral axotomy. Neuroscience 68:867–881.

81. Caligiorne, S. M., Santos, R. A. S., and Campagnole-Santos, M. J. (1996). Cardiovascular effects produced by bradykinin microinjection into the nucleus tractus solitarii of anaesthetized rats. Brain Res., 720:183–190.

82. Figueroa, C. D., Dietze, G., and Muller-Esterl, W. (1996). Immunolocalization of bradykinin B2 receptors on skeletal muscle cells. Diabetes 45 Suppl. 1:S24–S28.

83. Song, Q., Wang, D. Z., Harley, R. A., Chao, L., and Chao, J. (1996). Cellular localization of low-molecular-weight kininogen and bradykinin B2 receptor mRNAs in human kidney. Am. J. Physiol. 270:F919–F926.

84. Schremmer-Danninger, E., Heinz-Erian, P., Topfer-Petersen, E., and Roscher, A. A. (1995). Autoradiographic localisation and characterisation of bradykinin receptors in human skin. Eur. J. Pharmacol. 283:207–216.

85. Wiernas, T. K., Griffin, B. W., and Sharif, N. A. (1997). The expression of functionally-coupled B_2-bradykinin receptors in human corneal epithelial cells and their pharmacological characterization with agonists and antagonists. Br. J. Pharmacol. 121:649–656.

86. Chan, D., Gera, L., Helfrich, B., Helm, K., Stewart, J., Whalley, E., and Bunn, P. (1996). Novel bradykinin antagonist dimers for the treatment of human lung cancers. Immunopharmacol 33(1–3):201–204.

87. Nagy, I., Matesz, K., Woolf, C. J., and Urman, L. (1993). Cobalt uptake enables identification of capsaicin and bradykinin sensitive sub-populations of rat dorsal root ganglion cells in vitro. Neuroscience 56:167–172.

88. Babbedge, R., Dray, A., and Urban, L. (1995). Bradykinin depolarises the rat isolated superior cervical ganglion via B_2 receptor activation. Neurosci. Lett. 193:161–164.

89. Davis, A. J., and Perkins, M. N. (1994). Induction of B_1 receptors in vivo in a model of persistent inflammatory mechanical hyperalgesia in the rat. Neuropharmacol 33:127–133.

90. Perkins, M. N., Campbell, E., and Dray, A. (1993). Antinociceptive activity of the bradykinin B_1 and B_2 receptor antagonists, des-Arg⁹[Leu⁸]-BK and HOE 140, in two models of persistent hyperalgesia in the rat. Pain 53:191–197.

91. Perkins, M. N., and Kelly, D. (1993). Induction of bradykinin B_1 receptors in vivo in a model of ultra-violet irradiation-induced thermal hyperalgesia in the rat. Br. J. Pharmacol. 110:1441–1444.

92. Rupniak, N. M. J., Boyce, S., Webb, J. K., Williams, A. R., Carlson, E., Borkowski, J. A., Hess, J. F., and Hill, R. G. (1997). Effects of the bradykinin B_1 receptor antagonist des-Arg⁹[Leu⁸]bradykinin and genetic disruption of the B_1 receptor on nociception in rats and mice. Pain 71:89–97.

93. Cruwys, S. C., Garrett, N. E., Perkins, M. N., Blake, D. R., and Kidd, B. L. (1994). The role of bradykinin B_1 receptors in the maintenance of intra-articular plasma extravasation in chronic antigen-induced arthritis. Br. J. Pharmacol. 113:940–944.

94. Dray, A., and Perkins, M. N. (1993). Bradykinin and inflammatory pain. Trends Neurosci. 16:99–104.

95. Steranka, L. R., DeHaas, C. J., Vavrek, R. J., Stewart, J. M., Enna, S. J., and Snyder, S. H. (1987). Antinociceptive effects of bradykinin antagonists. Eur. J. Pharmacol. 136:261–262.

96. Heapy, C. G., Shaw, J. S., and Farmer, S. C. (1993). Differential sensitivity of antinociceptive assays to the bradykinin antagonist HOE 140. Br. J. Pharmacol. 108:209–213.

97. Costello, A. H., and Hargreaves, K. M. (1989). Suppression of carrageenan-induced hyperalgesia, hyperthermia and edema by a bradykinin antagonist. Eur. J. Pharmacol. 171:259–263.

98. Regoli D., Drapeau, G., Rovero, P., Dion, S., Rhaleb, N. E., Barabe, J., D'Orleans-Juste, P., and Ward, P. (1986). Conversion of kinins and their antagonists into B_1 receptor activators and blockers in isolated vessels. Eur. J. Pharmacol. 127:219–224.

99. Erdos, E. G. (1990). Some old and some new ideas on kinin metabolism. J. Cardiovasc. Pharmacol. 15:S20–S24.

100. Meini, S., Lecci, A., and Maggi, C. A. (1996). The longitudinal muscle of rat ileum as a sensitive monoreceptor assay for bradykinin B1 receptors. Br. J. Pharmacol. 117:1619–1624.

101. Correa, C. R., and Calixto, J. B. (1993). Evidence for participation of B_1 and B_2 kinin receptors in formalin-induced nociceptive response in the mouse. Br. J. Pharmacol. 110:193–198.

102. Shibata, M., Ohkubo, T., Takahashi, H., and Inoki, R. (1989). Modified formalin test: characteristic biphasic pain response. Pain 38:347–352.

103. Mantione, C. R., and Rodriguez, R. (1990). A bradykinin (BK)₁ receptor antagonist blocks capsaicin-induced ear inflammation in mice. Br. J. Pharmacol. 99:516–518.

104. Asano, M., Inamura, N., Hatori, C., Sawai, H., Kujiwara, T., Katayama, A., Kayakiri, H., Satoh, S., Abe, Y., Inoue, T., Sawada, Y., Nakahara, K., Oku, T., and Okuhara, M. (1997). The identifaction of an orally active, nonpeptide bradykinin B_2 receptor anatgonist, FR173657. Br. J. Pharmacol. 120:617–624.

105. Borkowski, J. A., Ransom, R. W., Seabrook, G. R., Trumbauer, M., Chen, H., Hill, R. G., Strader, C. D., and Hess, J. F. (1995). Targeted disruption of a B2 bradykinin receptor gene in mice eliminates bradykinin action in smooth muscle and neurons. J. Biol. Chem. 270(23):13706–13710.

106. Davis, A., and Perkins, M. N. (1993). The effect of capsaicin and conventional analgesics in two models of monoarthritis in the rat. Agents Actions 38 Spec. No:C10–C12.

107. Pesquero, J. B. (1997). Molecular characterisation and targeted disruption of the mouse B_1 receptor gene. Presented at Gordon Research Conference on Kallikreins and Kinins, Italy.

108. Alfie, M. E., Sigmon, D. H., Pomposiello, S. I., and Carretero, O. A. (1997). Effect of high salt intake in mutant mice lacking bradykinin B_2 receptors. Hypertension 29:483–487.

109. Alfie, M. E., Yang, X. P., Hess, F., and Carretero, O. A. (1996). Salt-sensitive hypertension in bradykinin B_2 receptor knockout mice. Biochem. Biophys. Res. Commun. 224:625–630.